GRAS Notice (GRN) No. 474
http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm

Soni & Associates Inc.

749 46th Square
Vero Beach, FL 32968, USA
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May 20, 2013

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Subject: GRAS Notification for Black Pepper Extract (Bioperine®)

Dear Sir/Madam:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Sabinsa Corporation, through Soni & Associates Inc. as its agent, hereby provides notice of a claim that the food ingredient black pepper extract (Bioperine®) described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be Generally Recognized As Safe (GRAS), based on scientific procedures.

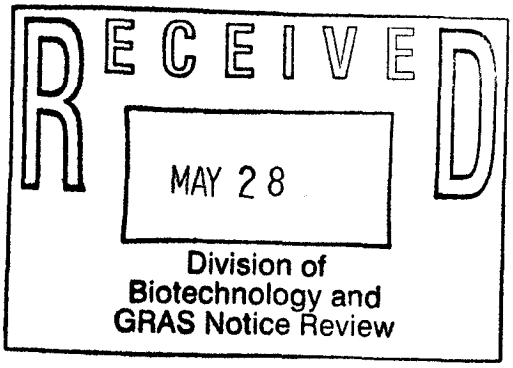
As required, please find enclosed three copies of the notification. If you have any questions or require additional information, please feel free to contact me by phone at 772-299-0746 or by email at sonim@bellsouth.net.

Sincerely,

(b) (6)

Madhu G. Soni, Ph.D., FATS

www.soniassociates.net



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GRAS NOTIFICATION

I. Claim of GRAS Status

A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR § 170.36(c)(1)

Sabinsa Corporation (the notifier) has determined that black pepper extract (BioPerine®) derived from fruits of *Piper nigrum* L (black pepper) or *P. longum* L (long pepper) is Generally Recognized As Safe, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use as a food ingredient. Therefore, the use of black pepper extract (BioPerine®) is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Date May 20, 2013

Madhu G. Soni, Ph.D., FATS

Agent for:

Sabinsa Corporation
20 Lake Drive
East Windsor, NJ 08520
USA

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Division of Biotechnology and GRAS Notice Review

B. Name and Address of Notifier:

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Vice President, Science and Technology
Sabinsa Corporation
20 Lake Drive
East Windsor, NJ 08520
USA

Tel: (732) 777-1111, Ext. 44
Fax: (732) 777-1443
Email: reza@sabinsa.com

C. Common or Usual Name of the Notified Substance:

The common name of the substance of this notification is black pepper extract. The preparation primarily contains piperine extracted from the fruits of *Piper nigrum* L (black pepper) or *P. longum* L (long pepper). The trade name of the substance is BioPerine®.

D. Conditions of Intended Use in Food

BioPerine®, an extract from black pepper, is intended for use as a flavoring agent (flavor enhancer) [21 CFR§170.3(o)(11)][1] in the following food categories: Baked Goods, Breakfast Cereals, Milk Products, Cheese, Egg Products, Processed Vegetables, Soft Candy, Soups, Snack Foods, Beverages Type I - Non-alcoholic, Imitation Dairy Products, Hard Candy, Chewing Gum, Granulated Sugar, Seasonings & Flavors) at use levels of up to 15 ppm. The extract is not proposed for uses in foods that are intended for infants and toddlers, such as infant formulas or foods formulated for babies or toddlers, as well as it is not intended for use in meat and poultry products that come under USDA jurisdictions. The intended use of BioPerine® in the above mentioned food categories, is estimated to result in a maximum daily intake of 13.70 mg/person/day (0.23 mg/kg body weight/day for an individual weighing 60 kg).

E. Basis for GRAS Determination:

In accordance with 21 CFR 170.30, the intended use of black pepper extract (BioPerine®) has been determined to be Generally Recognized As Safe (GRAS) based on scientific procedures. The determination is supported by the opinion of the Expert Panel. A comprehensive search of the scientific literature was also utilized for this determination. There exists sufficient qualitative and quantitative scientific evidence, including human and animal data to determine safety-in-use for black pepper extract (BioPerine®). The source material of the extract, black peppers has been used as foodstuff for centuries. The use of black pepper, its essential oil, oleoresin and natural extractives in food for human consumption is considered Generally Recognized As Safe (GRAS) by the FDA for use as a

[1]"Flavor enhancers": Substances added to supplement, enhance, or modify the original taste and/or aroma of a food, without imparting a characteristic taste or aroma of its own.

flavoring agent in foods. The safety determination of black pepper extract (BioPerine®) is based on the totality of available evidence.

The safety of BioPerine® is supported by multiple animal and human studies that have been performed with black pepper extract, oleoresin and piperine. Several experimental studies, including subchronic toxicity, *in vitro* and *in vivo* genotoxicity and human clinical safety data support the safety in use of black pepper extract at the intended use levels. Additionally, the safety of black pepper extract, including its active component piperine, is well established in the literature based on the dietary consumption of foods containing black pepper, its oleoresin and piperine. Furthermore, Joint FAO/WHO Expert Committee on Food Additives (JECFA) has determined that the available evidence for piperine supports acceptable daily intake (ADI) as "acceptable". On the basis of scientific procedures[2], Sabinsa Corporation considers the consumption of black pepper extract (BioPerine®), as a food ingredient to be safe at levels up to 13.70 mg piperine/person/day.

F. Availability of Information:

The data and information that forms the basis for this GRAS determination will be provided to Food and Drug Administration upon request or will be available for FDA review and copying at reasonable times at the above mentioned offices of the notifier (Section I, B) or at the offices of:

Madhu G. Soni, PhD, FATS
Soni & Associates Inc
749 46th Square
Vero Beach, FL 32068

Telephone: +1- 772-299-0746
Email: sonim@bellsouth.net

II. Detailed Information About the Identity of the Notified Substance:

BioPerine® is a standardized off-white powder prepared from the fruits of *Piper nigrum* L (black pepper) or *P. longum* L (long pepper). The extract has a characteristic odor and pungent taste. The active principle of BioPerine® is an alkaloid piperine (>95%) and is responsible for the pungent taste.

A. Chemical name:

Piperine: 1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine;
Piperidine,1-[(2E,4E)-5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl];
1-piperoylpiperidine;

[2] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

1,3-Benzodioxol-5-yl-1-oxo-2,4-pentadienyl-piperine; Bioperine
IUPAC Name: (2E,4E)-5-(1,3-benzodioxol-5-yl)-1-piperidin-1-ylpenta-2,4-dien-1-one

B. Trade Name:

The subject of this notification will be marketed as BioPerine®

C. Chemical Abstract Registry and other Number:

Piperine: 94-62-2; EINECS No. 202-348-0

D. Chemical Formula:

The empirical formula of piperine is $C_{17}H_{19}NO_3$

E. Structure:

The chemical structure of piperine is presented in Figure II-E.

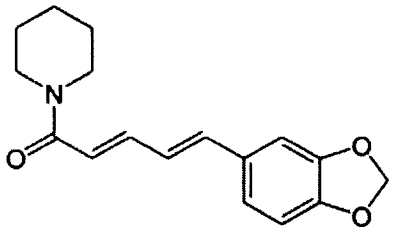

Figure II-E. Chemical Structure of piperine

F. Molecular Weight

The molecular weight of piperine is 285.34 Da

G. Physical Characteristics

BioPerine® is an off-white powder with a characteristic odor and pungent taste.

H. Typical Composition and Specifications

Typical food grade specifications of BioPerine® are presented in Tables II-H.1. Analytical data from five manufacturing lots prepared using extraction solvent ethyl alcohol are presented in Appendix I. General compositional analysis of BioPerine® is presented in Table II-H.2.

Table II-H.1. Specifications of black pepper extract (BioPerine®)

Parameter	Characteristics (Sabinsa, 2013*)	Method
Description	Off white to slight yellow powder	Visual
Identification	Comply with HPLC and IR spectrum	HPLC- In house
Piperine	Not less than 95% w/w and not more than 102% w/w on dry basis	HPLC- In house
Solubility	Soluble in alcohol and in glacial acetic acid; insoluble in water	USP
Loss on drying	NMT 2% w/w (dried at 105°C)	USP 731
Melting range	Between 126°C to 132°C	USP 741
Residue on ignition	NMT 0.1%	USP 281
Tapped bulk density	Between 0.55 and 0.85 g/mL	USP 616
Loose bulk density	Between 0.30 and 0.65 g/mL	USP 616
Sieve test (passes through)		
- 20 mesh	NLT 100%	
- 40 mesh	NLT 98%	
- 80 mesh	NLT 95%	
Heavy metals		
Lead	NMT 3 ppm	ICP-OES
Arsenic	NMT 1 ppm	ICP-OES
Cadmium	NMT 1 ppm	ICP-OES
Mercury	NMT 0.1 ppm	ICP-OES
Residual solvent	Comply with ICH/USP guidelines	ICH guidelines and USP
Residual pesticides	To comply with USP guidelines	USP 561
Microbiological assays		
Total plate count	< 3000 cfu/g	USP 2021
Yeast and Mold	< 100 cfu/g	USP 2021
Escherichia coli	Negative (cfu/10g)	USP 2021
Salmonella	Negative (cfu/10 g)	USP 2021
Staphylococcus aureus	Negative (cfu/10g)	USP 2021
Pseudomonas aeruginosa	Negative (cfu/10 g)	USP 2021
Enterobacteriaceae	Negative (cfu/10 g)	USP 2021

*Based on information provided by Sabinsa Corporation. NMT = Not more than; NLT = Not less than

Table II-H-2. Typical compositional analysis of BioPerine®

Parameter	
Piperine (alkaloid)	~98-99%
Other plant origin	~0.9-1.0%
Moisture	~0.2%
Inorganic matter (plant origin)	0.02%
Excipient/carrier	None
Extract ratio**	50:1
Ash	0.02%

*Based on information provided by Sabinsa Corporation; **On the basis of Black pepper dry fruits

I. Manufacturing process

BioPerine® is manufactured according to current good manufacturing practices (cGMPs) and this process is schematically presented in Figure II-I. The starting material for the preparation of BioPerine® is black pepper oleoresin that is prepared by solvent extraction of black pepper. The traces of solvent are removed by vacuum distillation at controlled temperature. The oleoresin is treated with ethanol at 50-60°C and then cooled to 10°C. The mixture is filtered and the precipitate thus obtained is dissolved in ethanol, treated with alumina at 50°C, cooled to 10°C and filtered to obtained crystals of piperine. The crystal of piperine thus obtained are washed with ethyl alcohol, dried, milled, passed through a sieve, and the resulting product is packaged. This process yields a powdered, purified product with over 95% piperine. The extraction procedure assures a consistent and high quality BioPerine® product.

J. Manufacturing process diagram

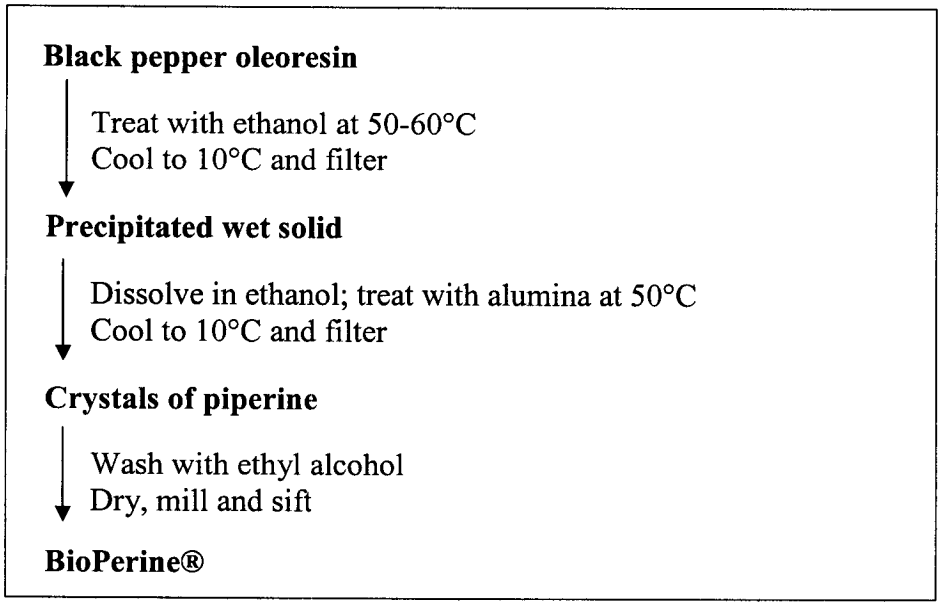

Figure II-I. Manufacturing process of BioPerine® (Sabinsa, 2013)

K. Intended Technical Effects

Black pepper extract primarily containing piperine is intended for addition to selected foods as a flavoring agent (flavor enhancer) [21 CFR§170.3(o)(11)][3] in the diet. The use of black pepper extract is intended for the general population at the levels identified in this document

[3]"Flavor enhancers": Substances added to supplement, enhance, or modify the original taste and/or aroma of a food, without imparting a characteristic taste or aroma of its own.

for addition to the following food categories: Baked Goods, Breakfast Cereals, Milk Products, Cheese, Egg Products, Processed Vegetables, Soft Candy, Soups, Snack Foods, Beverages Type I - Non-alcoholic, Imitation Dairy Products, Hard Candy, Chewing Gum, Granulated Sugar, Seasonings & Flavors. The intended use is at levels of up to 15 ppm. It is recognized that there are Standard of Identity requirements for some of these foods, and as such, Sabinsa Corporation does not intend to refer them by the commonly recognized names such as milk, or yogurt.

III. Summary of the Basis for the Notifier's Determination that Black Pepper Extract is GRAS

The determination that BioPerine® is GRAS is based on scientific procedures. A comprehensive search of the scientific literature for safety and toxicity information on black pepper, its oleoresin and piperine was conducted through February 2013 and was utilized for this assessment. Based on a critical evaluation of the pertinent data and information summarized here and employing scientific procedures, it is determined that the addition of black pepper extract (BioPerine®), primarily containing piperine, to the selected foods described in this notice and at use levels of 15 ppm meeting the specification cited above and manufactured according to current Good Manufacturing Practice, is GRAS under the conditions of intended use as specified herein.

In coming to this decision that black pepper extract is GRAS, Sabinsa Corporation relied upon the conclusions that neither black pepper extract nor any of its degradation products pose any toxicological hazards or safety concerns at the intended use levels, as well as on published toxicology studies and other articles relating to the safety of the product. Other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

IV. Basis for a Conclusion that Black Pepper Extract is GRAS for its Intended Use.

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened to determine the safety of BioPerine® used as a food ingredient. Based on a critical evaluation of the pertinent data and information summarized herein, the Expert Panel members have individually and collectively determined by scientific procedures that the addition of black pepper extract (BioPerine®) in Baked Goods, Breakfast Cereals, Milk Products, Cheese, Egg Products, Processed Vegetables, Soft Candy, Soups, Snack Foods, Beverages Type I - Non-alcoholic, Imitation Dairy Products, Hard Candy, Chewing Gum, Granulated Sugar, Seasonings & Flavors at use levels up to 15 ppm when not otherwise precluded by a Standard of Identity as described here and resulting in the maximum (90^{th} percentile) all-user estimated intake of 13.70 mg/person/day (0.23 mg/kg body weight/day for an individual weighing 60 kg) is GRAS. It is also their opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion (see attached Expert Panel Statement).

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF BLACK PEPPER EXTRACT (BIOPERINE®) AS A FLAVOR AGENT

Prepared by
Soni & Associates Inc.
749 46th Square
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Prepared for
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Panel Members

John A. Thomas, Ph.D., F.A.T.S., D.A.T.S.
James T. Heimbach, Ph.D., F.A.C.N.
Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S.

April 2013

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF BLACK PEPPER EXTRACT (BIOPERINE®) AS A FLAVORING AGENT

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DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF BLACK PEPPER EXTRACT (BIOPERINE®) AS A FLAVORING AGENT

1. INTRODUCTION

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[1], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by Soni & Associates Inc. at the request of Sabinsa Corporation, USA, to determine the Generally Recognized As Safe (GRAS) status of black pepper extract (BioPerine®) as a flavoring agent [21 CFR§170.3(o)(12)][2] in selected food products (Baked Goods, Breakfast Cereals, Milk Products, Cheese, Egg Products, Processed Vegetables, Soft Candy, Soups, Snack Foods, Beverages Type I - Non-alcoholic, Imitation Dairy Products, Hard Candy, Chewing Gum, Granulated Sugar, Seasonings & Flavors) at use levels of up to 15 ppm (15 mg/kg or 0.0015%). A comprehensive search of the scientific literature for safety and toxicity information on black pepper extract and its principal constituent piperine was conducted through February 2013 and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by Sabinsa Corporation and other information deemed appropriate or necessary. Sabinsa Corporation assures that all unpublished information in its possession and relevant to the subject of this determination has been provided to Soni & Associates Inc. and has been summarized accurately in this GRAS monograph. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

1.1. Background

Pepper is thought to have originated in the monsoon forests of the Malabar Coast in southwest India. From its earliest usage about 4000 years ago, pepper has reigned as the "King of spices" or "master spice." In addition to its uses in food, black pepper is used for other purposes such as medicinal, as a preservative, in perfumery, and even as an insecticide. Sanskrit literature from India that is considered as three thousand years old also mentions pepper. Pepper was one of the earliest items traded in Asia and Europe. During the 14th century, Vasco da Gama arrived on the west coast of India from Portugal with the intention of developing trade in spices, particularly black pepper (Ramasarma, 2000). Black pepper (*Piper nigrum*) is one of the most widely used among spices. In addition to its use as food, the ancient Greeks and Romans valued black pepper so highly it was used as a form of currency. Rome itself was once held hostage for a ransom of gold, silver and 3000 pounds of pepper. The cities of Alexandria, Genoa and Venice owed their economic success to pepper. During Medieval times, Europeans often used peppercorns as a currency to pay rent, dowries and taxes, and Shakespeare mentions pepper in his plays. In the 15th century, demand for pepper inspired Spanish exploration

[1]Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (curriculum vitae) documenting the expertise of the Panel members.

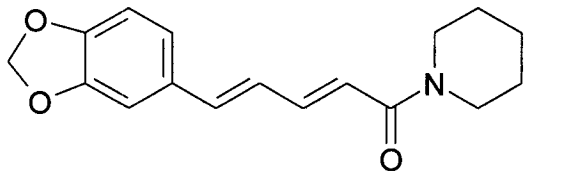
[2]*"Flavoring agents and adjuvants"*: Substances added to impart or help impart a taste or aroma in food.

and the spice trade. Peppercorns were sometimes swallowed whole as a general body tonic.

Piper is a genus of many species that grow in moist, warm parts of the world. The plant is a perennial vine cultivated in India, the Sunda Islands, Madagascar and the Comoro Islands. The plant exhibits a thick, branched stalk; alternate, spear-shaped leaves; sessile flowers arranged in spikes opposite the leaves; and round berries that turn from green to dark red on ripening. The vines take 7-8 years to reach maturity, after which they produce hundreds of spike flowers, each of which contains approximately 50 berries called black pepper. Black pepper is valued for its distinct biting quality attributed to the pungent alkaloid piperine and its isomers (Govindarajan, 1977). The best-known commercial pepper grades are reported to be Malabar and Lampong. Both have a less-developed aroma, but Lampong pepper is quite hot. Approximately 40% of the oleoresin of black pepper is piperine. In addition to *P. nigrum,* piperine is also found in *P. longum* Linn. *P. longum* L (long pepper) is a close relative of *P nigrum*, giving black, green and white pepper, and has a similar, though generally hotter, taste.

1.2. Description

The subject of this GRAS determination, BioPerine®, is a standardized extract prepared from the fruits of *Piper nigrum* L (black pepper) or *P. longum* L (long pepper).BioPerine® is an off-white powder with a characteristic odor and pungent taste. The principal active ingredient of BioPerine®, piperine (>95%), is responsible for the pungency. Piperine (Figure 1) is chemically known as 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine. General descriptive characteristics of BioPerine® are summarized in Table 1.



$C_{17}H_{19}NO_3$ Mol.wt. 285.34

Piperine

Figure 1. Chemical structure of piperine, the active principle of black pepper extract

Multiple varieties of pepper are grown with differences in the size of berry, length of spike, yield, chemical composition, etc. In the literature, varieties and cultivars of black pepper are referenced synonymously. Over 60 cultivars of black pepper are known to be cultivated in India (Gopalam and Zacharia, 1988). Whole dried berries, consisting of the epicarp, mesocarp and endocarp, yield black pepper. Berries from which covering such as the epicarp and the outer portion of the mesocarp have been removed by manual or mechanical maceration yield white pepper. The epicarp contains the resinous fraction consisting of chavicin and the outer portion of the mesocarp contains the majority of the

essential oil. The resinous fraction and essential oil have their characteristic aromas, while white pepper has little aroma. Thus, black pepper yields an essential oil and oleoresin, while white pepper contains piperine. The sharp, piquant flavor of white pepper is due to the presence of piperine. Although white pepper and black pepper are obtained from the same species of *P. nigrum*, FDA and FEMA consider each separately with separate CAS numbers and FEMA numbers (EAFUS, 2008; Hall and Oser, 1965).

Table 1. General descriptive characteristics of BioPerine®

Parameter	**Description (Sabinsa, 2013)***
Botanical source	*Piper nigrum* L. or *Piper longum* L.
Botanical family	Piperaceae
Plant part used	Fruit/Berries
Synonym	Black pepper extract
Appearance	Powder
Color	Off-white to slight yellow
Odor	Characteristic
Taste	Pungent
Storage	Store at room temperature (15-25°C)
Stability	5 years
Functional use in food	Flavoring agent and adjuvant
Active ingredient	Piperine
Chemical name	Piperidine, 1-[(2E,4E)-5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]; 1-piperoylpiperidine; 1,3-Benzodioxol-5-yl-1-oxo-2,4-pentadienyl-piperine
IUPAC Name	(2E,4E)-5-(1,3-benzodioxol-5-yl)-1-piperidin-1-ylpenta-2,4-dien-1-one
CAS No.	94-62-2
EINECS No.	202-348-0
Molecular weight	285 Daltons
Chemical formula	$C_{17}H_{19}NO_3$

*Based on information provided by Sabinsa Corporation

1.3. Specifications and Identity

Food grade specifications and typical compositional analysis of BioPerine® from Sabinsa Corporation are presented in Tables 2 and 3, respectively. Analytical results from five non-consecutive lots (Appendix I) demonstrate that BioPerine® meets the standard specifications. BioPerine® is freely soluble in alcohol, benzene and glacial acetic acid and is insoluble in water. The residual solvent levels for ethanol used in the manufacturing of BioPerine® comply with ICH/USP guidelines. The residual solvent levels (i.e., ethanol, hexane, ethyl acetate, and methanol) from four lots are presented in Appendix II.

Table 2. Specifications of black pepper extract (BioPerine®)

Parameter	Characteristics (Sabinsa, 2013*)	Method
Physical form and color	Off white to slight yellow powder	Visual
Identification	Comply with HPLC and IR spectrum	HPLC- In house
Piperine	Not less than 95% w/w and not more than 102% w/w on dry basis	HPLC- In house
Solubility	Soluble in alcohol and in glacial acetic acid; insoluble in water	USP
Loss on drying	NMT 2% w/w (dried at 105°C)	USP 731
Melting range	126°C to 132°C	USP 741
Residue on ignition	NMT 0.1%	USP 281
Tapped bulk density	Between 0.55 and 0.85 g/mL	USP 616
Loose bulk density	Between 0.30 and 0.65 g/mL	USP 616
Sieve test (passes through)		
- 20 mesh	NLT 100%	
- 40 mesh	NLT 98%	
- 80 mesh	NLT 95%	
Heavy metals		
Lead	NMT 3 ppm	ICP-OES
Arsenic	NMT 1 ppm	ICP-OES
Cadmium	NMT 1 ppm	ICP-OES
Mercury	NMT 0.1 ppm	ICP-OES
Residual solvent	Complies with ICH/USP guidelines	ICH guidelines and USP
Residual pesticides	To comply with USP guidelines	USP 561
Microbiological assays		
Total plate count	< 3000 cfu/g	USP 2021
Yeast and Mold	< 100 cfu/g	USP 2021
Escherichia coli	Negative (cfu/10g)	USP 2021
Salmonella	Negative (cfu/10 g)	USP 2021
Staphylococcus aureus	Negative (cfu/10g)	USP 2021
Pseudomonas aeruginosa	Negative (cfu/10 g)	USP 2021
Enterobacteriaceae	Negative (cfu/10 g)	USP 2021

*Based on information provided by Sabinsa Corporation. NMT = Not more than; NLT = Not less than

Table 3. Typical compositional analysis of BioPerine®

Parameter	
Piperine (alkaloid)	~95-99%
Other plant origin	~1.0-5.0%
Moisture	~0.2%
Inorganic matter (plant origin)	0.02%
Excipient/carrier	None
Extract ratio**	50:1
Ash	0.02%

*Based on information provided by Sabinsa Corporation;
**On the basis of Black pepper dry fruits

1.4. Manufacturing Process

BioPerine® is manufactured according to current good manufacturing practices (cGMPs) and this process is schematically presented in Figure 2. The starting material for the preparation of BioPerine® is black pepper oleoresin that is prepared by solvent extraction of black pepper. The traces of solvent are removed by vacuum distillation at controlled temperature. The oleoresin is treated with ethanol at 50-60°C and then cooled to 10°C. The mixture is filtered and the precipitate thus obtained is dissolved in ethanol,

treated with alumina at 50°C, cooled to 10°C and filtered to obtained crystals of piperine. The crystals of piperine thus obtained are washed with ethyl alcohol, dried, milled, passed through a sieve, and packaged. The manufacturing procedure assures a consistent and high quality BioPerine® product.

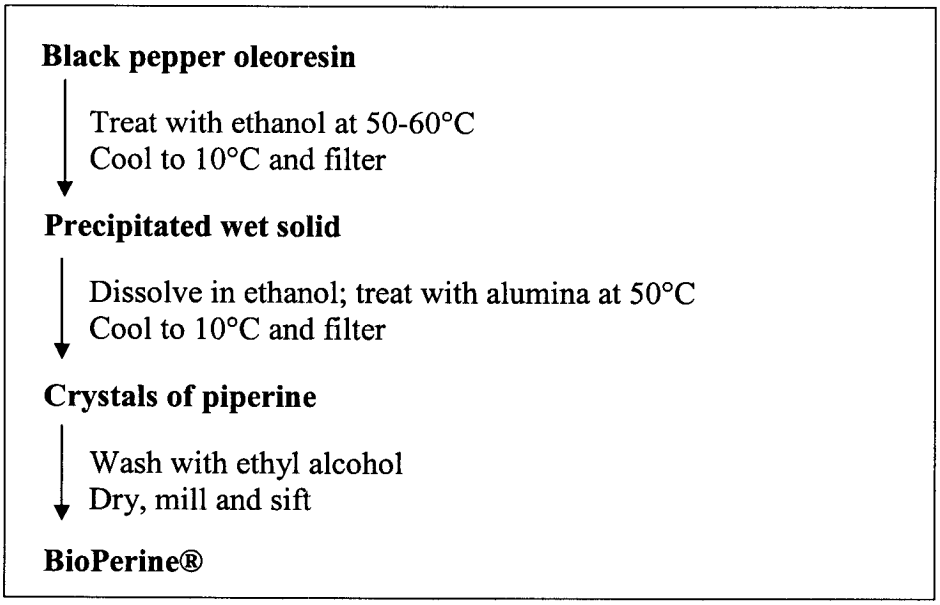


Figure 2. Manufacturing process of BioPerine® (Sabinsa, 2013)

1.5. Current Uses

Traditionally, black pepper has been used as a foodstuff, a cosmetic, and for therapeutic purposes. As a spice, black pepper is used in curry to provide its distinctive flavor. Essential oil isolated from black pepper is extensively used as a flavor component in many food products including alcoholic and non-alcoholic beverages. The highest maximum use level for pepper oils in food products is about 0.04%. Black pepper oil is generally used as a flavor modifier for other spices where pungency is not needed. It is utilized as a flavor ingredient in several food products, *e.g.* in alcoholic beverages, baked goods, condiment/relish, gelatins/puddings, meat products and soups. Black pepper oil is used with eugenol and isoeugenol in carnation and rose bases and in oriental fragrances. Black pepper oil is used in soaps, detergents, creams/lotions and perfumes. Black pepper is commonly used as a stimulant, carminative and tonic. It is also used for colds, aches and pains, influenza, flatulence and rheumatism. Piperine has been claimed to reduce inflammation, improve digestion, and relieve pain and asthma (Singh and Duggal, 2009). Black pepper is used for digestive disorders and is recommended for neuralgia and scabies. It is also used in aromatherapy. Piperine is used as a flavoring substance and adjuvant.

1.6. Regulatory Status

The Flavor and Extract Manufacturers' Association (FEMA No. 2909) has approved use of black pepper (FEMA No. 2844), black pepper oil (FEMA No. 2845), black pepper oleoresin (FEMA No. 2846), and piperine (FEMA No. 2909) as GRAS substances (Hall and Oser, 1965). The use levels of black pepper oil cited by FEMA are 2

ppm in processed vegetables, 25 ppm in baked goods, and 170 ppm in meat products (Hall and Oser, 1965). The maximum FEMA cited use levels of black pepper oleoresin, which contains approximately 40% piperine, are: beverages 25 ppm; ice cream/ices 20 ppm; candy 15 ppm; baked goods 1600 ppm; condiments 370 ppm; and meats 230 ppm. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated the use of piperine (JECFA No. 1600) as a food additive and established an acceptable daily intake (ADI) as "acceptable." JECFA determined that there is no safety concern at current levels of intake of piperine when used as a flavoring agent (JECFA, 2005). At its 65th Meeting, JECFA prepared specification for piperine and adopted them from the year 2006. The regulatory status of piperine and black pepper by different agencies is summarized in Table 4.

FDA has recognized black pepper, its essential oil, oleoresin and natural extractives as GRAS for their intended use. Additionally, synthetic piperine is on the FDA's list of Food Additives Permitted for Direct Addition to Food for Human Consumption as a synthetic flavoring substance and adjuvant (21 CFR 172.515). As a synthetic flavoring substance and adjuvant, piperine may be safely used in food in accordance with the following conditions: It is used in the minimum quantity required to produce its intended effect, and otherwise in accordance with all the principles of good manufacturing practice; and it can be used alone or in combination with flavoring substances and adjuvants GRAS in food, prior-sanctioned for such use, or regulated by an appropriate section of 21 CFR 172.515. Although these CFR citations are for synthetic piperine, as indicated earlier BioPerine® is prepared from black pepper and contains >95% piperine.

Table 4. Regulatory status of piperine and black pepper

Agency	Citation/Comments	Food category	Permitted functionality	Use limits
FDA	Piperine: 21 CFR 172.515 Food Additives Permitted for Direct Addition to Food for Human Consumption as a synthetic flavoring substance and adjuvant. Black pepper oil: 21 CFR 182.20 Substances generally recognized as safe – Essential oils, oleoresin (solvent-free), and natural extractives (including distillates)	No restrictions	Flavoring agents and adjuvants	cGMP
FEMA	Piperine: 2909, GRAS Black pepper oil: 2845, GRAS		Flavor ingredient	
JECFA	Piperine: 1600, No safety concern at current levels of intake when used as a flavoring agent		Flavor ingredient	ADI= acceptable

1.7. Technical Effects

The intended uses of BioPerine® are for addition as a flavoring agent [21 CFR§170.3(o)(12)][3] to selected foods. Because of its characteristic pungent flavor, use of BioPerine® influences the sensory perception of food. The pungent taste of the piperine serves to limit its intake. Given the pungent taste of both black pepper and BioPerine®, it is unlikely that both ingredients will be used in the same foods at the usual

[3] *"Flavoring agents and adjuvants"*: Substances added to impart or help impart a taste or aroma in food.

levels of individual ingredients. This also suggests that the intake of piperine is unlikely to be additive from its natural presence in pepper and from the proposed addition to food.

1.8. Intake from Natural Presence in Food

The primary sources of dietary exposure to piperine are black pepper, and its oil and oleoresin. The piperine content of black pepper varies from 5 to 9%. Dietary consumption of black pepper varies considerably from one population group to another and even within a population group. Kindell (1984) reported that in the United States the average daily consumption of black pepper was 359 mg. Given that the content of piperine in black pepper varies between 5 and 9%, this would suggest a daily consumption of approximately 18 to 32 mg of piperine. In addition to black pepper, its essential oil and oleoresin are utilized as flavor ingredients in alcoholic beverages, baked goods, beverages, condiments/relishes, gelatins/puddings, meat products and soups. The reported use levels of black pepper oil are 2.7 ppm in beverages, 20 ppm in ice-cream/ices, 5.3 ppm in candy, 8.5 ppm in baked goods, 17 ppm in candy, and 140 ppm in meat products (Hall and Osher, 1965). Similarly, reported use levels of black pepper oleoresin in different food categories are: 15 ppm in beverages, 20 ppm in ice-cream/ices, 15 ppm in candy, 1600 ppm in baked goods, 370 ppm in candy and 230 ppm in meat products (Hall and Osher, 1965). Both black pepper oil and oleoresin are reported to contain piperine at levels of up to 5 and 40%, respectively. These observations suggest a considerable dietary exposure of piperine. Although black pepper oil and oleoresin are used to replace black pepper and the pungent taste of the active ingredient piperine may limit its uses, it is likely that use of oleoresin may result in higher intake of piperine compared to that from use of black pepper or black pepper oil.

Synthetic piperine is approved as a direct food additive (21CFR 172.515). It is reported in foods at concentrations ranging from 0.4 to 6 ppm in candy up to 640 ppm in baked goods. In one report, the estimated human dietary consumption of piperine was reported as 0.3 to 0.54 mg/kg bw/day (Fenaroli, 1974). In this report the values for piperine were calculated based on data showing that the oleoresin of black pepper is 40% piperine. For an individual weighing 60 kg, the average daily intake of piperine is 32 mg/day.

1.9. Intended Use Levels and Food Categories

Sabinsa Corporation intends to use BioPerine®, as a flavoring agent at use levels of up to 15 ppm in Baked Goods, Breakfast Cereals, Milk Products, Cheese, Egg Products, Processed Vegetables, Soft Candy, Soups, Snack Foods, Beverages Type I - Non-alcoholic, Imitation Dairy Products, Hard Candy, Chewing Gum, Granulated Sugar, Seasonings & Flavors (Table 5). Although some foods with standards of identity are included in the list of foods, at present the use of BioPerine® is intended for foods without a standard of identity.

1.9.1. Estimated Daily Intake from the Intended Uses

Estimates of the daily intake of BioPerine® expected to result from its maximum intended use levels have been determined using the mean consumption estimates of designated food categories based on Market Research Corporation of America (MRCA) mean frequency of eating and USDA mean portion size of 34 general food categories

data (MRCA, 1965). In addition to intake surveys by USDA, marketing research groups such as MRCA and the National Purchase Diary (NPD) have also surveyed the food consumption patterns of individuals and households. The primary purpose of these surveys is to determine the nutritional adequacy of diets rather than the safety of food with respect to additives or contaminants. However, these surveys are frequently used to assess exposure to additives and contaminants. Under an FDA contract, the Federation of American Societies for Experimental Biology (FASEB, 1988) reviewed the theory behind the calculation of exposure estimates from different surveys including the MRCA. FDA historically relied on MRCA survey data to determine consumption estimates.

Although the MRCA method was developed in 1965, it is still accepted by the FDA in determining the possible average daily intake of food ingredients (DiNovi and Kuznesof, 1995). The conservatism of this determination method assumes that the maximum amount of substance is added to the entire food category, not just the food product within that category. The intake represents per capita rather than per user. Given the intended uses of BioPerine® in multiple widely consumed food categories, per capita intake are closer to per user intake as nearly everyone in the population is a user of at least one of these foods on a given day. Using MRCA estimated mean intakes of the food categories (Baked Goods, Breakfast Cereals, Milk Products, Cheese, Egg Products, Processed Vegetables, Soft Candy, Soups, Snack Foods, Beverages Type I - Non-alcoholic, Imitation Dairy Products, Hard Candy, Chewing Gum, Granulated Sugar, Seasonings & Flavors) for which BioPerine® is proposed to be added, the daily intake of BioPerine® from each of the categories is summarized in Table 5. The figures in Table 5 represent average per capita intakes, but the total estimated intake may be regarded as a per user intake since nearly everyone in the population is a user of at least one of these foods.

Table 5. Intended use levels and possible daily intake of BioPerine®*

Food category	Mean consumption of food product (g/day)	Use levels (ppm)	Daily intake by adult (mg/person)
Baked Goods	137.2	15	2.058
Breakfast Cereals	20	15	0.300
Milk Products	39.5	15	0.600
Cheese	9.4	15	0.140
Egg Products	1.9	15	0.180
Processed Vegetables	85	15	1.270
Soft Candy	5.8	15	0.100
Soups	31.7	15	0.470
Snack Foods	1.3	15	0.020
Beverages Type I - Non-alcoholic	104	15	1.560
Imitation Dairy Products	0.9	15	0.010
Hard Candy	0.6	15	0.010
Chewing Gum	0.2	15	0.001
Granulated Sugar	8.6	15	0.130
Seasonings & Flavors	0.01	15	0.001
		Total (mg/person/day)	**6.850**

*The daily intake calculations are based on MRCA (1965) data on mean frequency of eating and USDA report on mean portion size

Analysis of the results of projected consumption and thus exposure revealed that the intended uses of BioPerine® in the specified food categories result in a mean estimated daily intake of 6.85 mg BioPerine®/person. In order to estimate the 90th percentile consumption of BioPerine®, the corresponding mean total intake value from all food categories was multiplied by two on the grounds that the 90th percentile consumption may generally be estimated as two times the mean (FDA, 2006). These assumptions and the analysis above indicate that the mean and 90th percentile estimated daily intake of the BioPerine ® from its intended food uses is 6.85 and 13.70 mg/person/day or 0.11 and 0.23 mg/kg body weight/day, respectively (considering an average body weight of 60 kg).

2. TOXICOLOGY

Several experimental studies support the safety-in-use of black pepper and its preparations. The majority of the experimental *in vitro,* animal or human studies conducted with black pepper or its preparations, including piperine, were undertaken to evaluate its efficacy for different health conditions. A comprehensive search of the scientific literature for safety and toxicity information did not reveal any significant adverse effects of black pepper or its preparations at the commonly used levels. As BioPerine® primarily contains piperine, the present assessment focuses on safety of piperine. National and international agencies such as FDA, EFSA and JECFA have extensively reviewed safety data on piperine.

2.1. Absorption, Distribution and Excretion

Bhat and Chadrasekhara (1986a) investigated the absorption, tissue distribution, and elimination of piperine in rats. In this study, groups of male albino Wistar rats (4-week-old weighing 59-65 g) were given piperine at a dose of 170 mg/kg bw by gavage or 85 mg/kg bw by intraperitoneal injection, and urine and feces were collected every 24 hours for over 12 days. Urine and feces samples from rats fed a control diet for 10 days were collected for 3 days before treatment and used as control. Irrespective of the route of administration, approximately 3% of the unchanged dose was detected in feces over 5 days. Peak excretion of piperine in the feces was noted on day one following intraperitoneal administration and on day three after gavage. No unchanged piperine was detected in urine following administration by either route. However, there was increased excretion of conjugated glucuronides, sulfates, and phenols, with maximum levels on days 1-4. Overall, 91-97% of the administered dose was accounted for. In additional experiments, following gavage treatment with piperine rats were killed at various intervals; blood was collected from the heart and tissues such as liver, kidney, spleen and gut (stomach, small intestine, caecum and large intestine) were collected. After 30 minutes of oral ingestion, 29% of administered piperine was detected in the gut (22% in stomach and 6% in small intestine). At the end of 48 hours, 1% was detected in stomach, and 2-3% in the caecum and large intestine, indicating that 97% had been absorbed. Following intraperitoneal injection, a similar pattern was noted, although some of the values differed (data not reported). Only traces of piperine were noted in blood collected between 1 and 10 hours following the administration by either route. Between 0.5 and 24 hours after treatment, intraperitoneally administered piperine was detected in the liver

(0.40-2.12%) and kidney (0.04-0.20%). Similarly, orally administered piperine was detected in the liver (0.12-0.25%) and kidney (0.03-0.17%) up to 24 hours after treatment. No piperine was detected after 48 hours in any of the tissues examined.

Bajad et al. (2002) measured the plasma time profile of piperine in rats. In this study, jugular vein canulated rats (n=5) were orally administered 20 mg piperine/kg bw and blood was collected at different time points for up to 8 hours. Piperine was detected in plasma from 0.25 to 8 hours following administration, with maximum plasma concentration (2.83 mg/ml) at 0.5 hours post dosing. The results of this study suggest that piperine is rapidly absorbed through the gastrointestinal tract and could be detected in plasma as early as 15 minutes after administration.

Bhat and Chandrasekhara (1987) also investigated urinary and biliary elimination of piperine in rats. In this study, a group of male albino Wistar rats (4-week-old weighing 59-65 g) were orally (gavage) administered with piperine at a dose level of 170 mg/kg bw. Some treated and control rats received bile duct cannulae and bile was collected for 6 hours. Urine was collected from the remaining rats for 4 days and pooled, while urine collected for 4 days before dosing served as control samples. No unchanged piperine was detected in urine. Piperic acid was detected in the bile (about 1% of the original dose) within 6 hours, and various metabolites (piperonylic acid, piperonal, vanillic acid, and piperonyl alcohol) were excreted in urine (about 15.5% of the original dose) within 96 hours.

In its evaluation of a group of structurally related aliphatic and aromatic amides used as flavoring ingredients, including piperine, JECFA (2005) reported that studies on selected members of the group indicate that amides per se are rapidly absorbed and metabolized.

2.1.1. Metabolism and Bioavailability

Aliphatic amides, the group to which piperine belongs, have been reported to undergo limited hydrolysis. In an early study, Bray et al. (1949) investigated hydrolysis of aliphatic amides of various lengths following incubation with rabbit liver extracts. Hydrolysis was significantly slower for aliphatic amides with fewer than five or more than 10 carbons (Bray et al., 1949). In the above described study (Section 2.1) by Bhat and Chandrasekhara (1986a), metabolism of piperine was also investigated. Although no unchanged piperine was detected in urine after exposure by either route, increased excretion was observed of conjugated glucuronides, sulfates and phenols, with maximum excretion of all three on days 1-4. Demethylenation of piperine was suggested by an increase in conjugated phenols. Over 8 days, about 36% of the gavage dose was excreted in urine as conjugated phenols and 62% as methylenedioxyphenyl metabolites. About 19% of the intraperitoneal dose was excreted as phenolics and about 72% as methylenedioxyphenyl derivatives. The results of these investigations indicate that amide hydrolysis products are not major metabolites of piperine hydrolysis (Bhat and Chandrasekhara, 1986a).

In a subsequent study, Bhat and Chandrasekhara (1987) investigated the metabolism of piperine in male Wistar rats following a single oral gavage dose of 170 mg/kg bw (suspended in 10% alcohol and subsequently in peanut oil). Urinary and biliary metabolites were evaluated by employing TLC, HPLC, and GC-MS. Piperonal

was not detected in bile, but was detected in 0-96 hour urine collection samples, representing approximately 1.3% of the administered dose. The authors speculated that piperonal is further metabolized to piperonyl alcohol (3.6% of the administered dose) and piperonylic acid (6.3% of the administered dose) and subsequently conjugated for excretion. The results of this study indicate that, in addition to amide hydrolysis leading to piperic acid, metabolic oxidative cleavage of the benzylic alkene function results in a series of vanilloyl and piperonyl derivatives, which are excreted free or in conjugated form, mainly in the urine.

Effects of piperine on drug metabolizing enzymes have been investigated in a number of studies. In *in vitro* studies, piperine was found to inhibit metabolizing activity of several rat hepatic cytochrome P450 (CYP) isoforms, including CYP1A (Atal et al., 1985), as well as glucuronidation by guinea pig enterocytes (Singh et al., 1986) with *K* values of 30 to 70 μM. Available studies demonstrate that piperine is a nonspecific inhibitor of drug metabolism which shows little discrimination between different CYP forms. Piperine was found to inhibit the hepatic arylhydrocarbon hydroxylase (AHH) and UDP-glucuronyltransferase activities. The maximal inhibition of AHH was observed within 1 hour and was restored to normal within 6 hours. These findings suggest that the piperine-induced inhibition of CYPs is transient.

Kang et al. (1994) reported that intraperitoneal administration of piperine to rats daily for 3 days at a dose level of 400 mg/kg bw/day also resulted in the inhibition of CYP2E1, but induced CYP1A and CYP2B activities. Shoba et al. (1998) investigated the effects of piperine administration on curcumin pharmacokinetics in Wistar rats. Co-administration of piperine (20 mg/kg bw) to rats receiving a curcumin dose of 2000 mg/kg bw resulted in increased bioavailability of curcumin by 154%. In another study in mice, Lambert et al. (2004) reported that co-administreation of piperine (70 μmol/kg bw; 20 mg/kg bw) enhanced the bioavailability of (-)-epigallocatechin-3-gallate (164 μmol/kg bw; 75 mg/kg bw) in male CD-1 mice by about 30% possibly by inhibiting glucuronidation and gastrointestinal transit. In human clinical investigations, CYP-inhibition has been reported following ingestion of piperine. In a review article, Wilkinson (1997) reported that piperine (20 mg/day) treatment for 7 days increased the oral bioavailability of propranolol and theophylline by about 100% and a similar increase in plasma levels was also noted with phenytoin.

In an *in vitro* study using human liver tissue microsomes, Volak et al. (2008) investigated the potential effects of piperine and curcumin on cytochrome P450, UDP-glucuronosyltransferase (UGT), and sulfotransferase (SULT) enzymes. The results of this study revealed that, compared to curcumin, piperine was a relatively selective noncompetitive inhibitor of CYP3A, with less effect on other enzymes evaluated (IC_{50} > 29 μM) such as SULT, CYP2C19, CYP2B6, UGT, and CYP2C9. Based on the data and expected tissue concentrations of these ingredients, Volak et al. (2008) predicted that orally administered curcuminoid/piperine combination is most likely to inhibit CYP3A, CYP2C9, UGT, and SULT metabolism within the intestinal mucosa. Interestingly, acetaminophen sulfation and glucuronidation activities were not affected by piperine at concentrations up to 50 μM.

In a crossover human clinical trial, Bano et al. (1991) investigated the effects of piperine on the bioavailability and pharmacokinetics of propranolol and theophylline. In

this study, six subjects in each group received a single oral dose of propranolol (40 mg) or theophylline (150 mg) alone or in combination with piperine 20 mg daily for 7 days. In the subjects receiving piperine and propranolol, an earlier Tmax and a higher Cmax and area under curve (AUC) were noted. With theophylline, piperine produced a higher Cmax, longer elimination half-life and a higher AUC. The investigators suggested that in clinical practice, the enhanced systemic availability of oral propranolol and theophylline could be exploited to achieve better therapeutic control and improved patient compliance.

Piperine has been claimed to enhance the bioavailability of several other compounds, including phenytoin, coenzyme Q10, β-carotene, theophylline, and propranolol (Bano et al., 1991; Badmaev et al., 1999; Badmaev et al., 2000; Pattanaik et al., 2006). Several mechanisms have been postulated for piperine's bioavailability-enhancing effect, including the formation of apolar complexes with other compounds, inhibition of efflux transport, and inhibition of gut metabolism (Atal et al., 1985; Khajuria et al., 1998; Bhardwaj et al., 2002). In the majority of these cases, piperine was co-administered with the other ingredient. Piperine has also been reported to inhibit p-glycoprotein (Bhardwaj et al., 2002). Similarly, several other herbal constituents such as ginsenosides, silymarin from milk thistle and other flavonoids, capsaicin, and resveratrol have also been reported to inhibit p-glycoprotein activity *in vitro* (Marchetti et al., 2007). The majority of the examples of herb-drug interactions are minor or theoretical at present. The modulation of metabolism by herbal constituents and its implications for drug therapies remain to be fully investigated from a mechanistic perspective. This area of research is the subject of several current investigations. The intended use of BioPerine® is unlikely to significantly affect the absorption of other substances for the following reasons: (1) co-administration has generally been shown to be necessary for increased absorption; (2) the intended uses result in 90th percentile intake of BioPerine® of 13 mg/day while in most investigational studies the dose of piperine producing effects on absorption was significantly higher (~20 mg/day); (3) ingestion of BioPerine® in foods is spread over a day rather than occurring as bolus doses such as those used in experimental studies; and (4) finally, piperine induced inhibition of CYPs is temporary or transient.

In a recent randomized placebo-controlled six-way crossover trial in eight healthy volunteers (Volak et al., 2013), a standardized curcuminoid/piperine preparation (4 g curcuminoids plus 24 mg piperine) or matched placebo was given orally four times over 2 days to subjects before oral administration of midazolam (CYP3A probe), flurbiprofen (CYP2C9 probe) or paracetamol (acetaminophen) (dual UGT and SULT probe). Plasma and urine concentrations of drugs, metabolites and herbals were measured by HPLC. Compared with placebo, the curcuminoid/piperine treatment produced no meaningful changes in plasma Cmax, AUC, clearance, elimination half-life or metabolite levels of midazolam, flurbiprofen or paracetamol (α = 0.05, paired t-tests). The investigators concluded that short term use of this piperine-enhanced curcuminoid preparation is unlikely to result in a clinically significant interaction involving CYP3A, CYP2C9 or the paracetamol conjugation enzymes.

2.2. Acute and Short-term Studies

In a series of investigations, Piyachaturawat et al. (1983) investigated acute and short-term effects of piperine in rodents. The LD_{50} values for a single intravenous, intraperitoneal, subcutaneous, intragastric and intramuscular administration of piperine in

adult male mice were 15, 43, 200, 330, and 400 mg/kg body weight, respectively. Compared to the adult male mice, the intraperitoneal LD_{50} value was higher in adult females (60 mg/kg) and in weanling male mice (132 mg/kg bw). In adult female rats, the intraperitoneal LD_{50} value was 33.5 mg/kg bw whereas the intragastric LD_{50} value was increased to 514 mg/kg bw. The majority of the animals given a lethal dose died of respiratory paralysis within 3-17 minutes.

In the short-term toxicity studies, groups of eight adult female Fischer rats were given piperine via gavage at a dose of 0 (solvent control), 100, 250, 350 or 500 mg/kg bw/day for 7 days (Piyachaturawat et al., 1983). A dose-related decrease in body weight gain and increase in the number of deaths was noted. Although no deaths occurred at the two lower doses, a slight reduction in body-weight gain (statistical significance not reported) was reported at 250 mg/kg bw/day. Two and five rats died after 1-3 days of treatment in the groups receiving 350 and 500 mg/kg bw/day, respectively. Necropsy of rats at 500 mg/kg bw/day revealed severe hemorrhage and edema of the gastrointestinal tract (stomach, small and large intestine). Hemorrhage in the urinary bladder also was reported in some rats. At the dose level of 250 mg/kg bw/day, three of eight rats had hemorrhage in the stomach. Histological examination of tissues showed changes in the stomach, urinary bladder, adrenal glands and small intestine. In the stomach, the main changes seen in rats receiving 500 mg/kg bw/day dose were hemorrhagic erosion and ulceration in the mucosa of the glandular portio. Severe hemorrhagic edema was also reported in the submucosa of the glandular and squamous portions. Gastric ulceration was noted in all animals receiving 500 mg/kg bw/day dose, while in groups receiving 250 and 350 mg/kg bw/day dose three and four showed gastric ulceration, respectively. Variations similar to those observed in the stomach were seen in the urinary bladder (doses not specified), consisting of degenerative lesions and hemorrhagic necrosis of the epithelial cell lining, accompanied by extremely severe hemorrhagic edema in the submucosa and muscular layers. Mild-to-moderate enteritis with epithelial cell necrosis and desquamation were reported in the small intestine of rats at the highest dose. Additionally, signs of severe hemorrhage and degenerative necrosis of the adrenal medulla, varying in degree of severity among rats, were reported at the highest dose. Mild fatty infiltration in the liver and cell necrosis in the corpora lutea were the only other significant histopathological changes reported (Piyachaturawat et al., 1983). No adverse effects were noted at 100 mg/kg bw/day.

In an extensive and critical review article on black pepper and its pungent principle-piperine, the author (Srinivasan, 2007) stated, "Earlier studies in our laboratory indicated that no adverse effect was caused by feeding black pepper or piperine at levels equivalent to normal human intake or as much as 250 times as indicated by growth, organ weights, and blood constituents (Srinivasan and Satyanarayana, 1981)." Additional details of the study were not available.

2.3. Subchronic Studies

Bhat and Chandrasekhara (1986b) investigated effects of black pepper, its oleoresin and piperine in weanling rats. In this study, groups of six male Wistar rats (4-week-old weighing 59-65 g) were fed diets containing black pepper oleoresin at a concentration of 110, 220 and 440 ppm, pepper at 2000 ppm, or piperine at 100 ppm for a period of 8 weeks. The dietary levels of pepper and oleoresin were intended to be

approximately 5-20 times the normal daily intake of humans. Given that the piperine content of the black pepper oleoresin was approximately 45%, the dietary levels of piperine resulting from feeding of the oleoresin were approximately 50, 100 and 200 ppm. These levels of piperine in pepper oleoresin (45% piperine) were calculated to provide average daily intakes of approximately 5, 10 and 20 mg/kg bw/day of piperine, respectively. The 100 ppm dietary level of piperine was calculated to provide an average daily intake of approximately 10 mg/kg bw. A group of control animals was maintained on basal diet. Ingestion of black pepper oleoresin, pepper or piperine had no effect on food intake, feed use efficiency, organ weights (liver, kidney, spleen, and adipose tissue), hematological parameters (hemoglobin, red blood cells, white blood cells, lymphocytes, and neutrophils) or clinical chemistry values (total protein, albumin:globulin ratio, glucose, cholesterol, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase). Isolated, non-dose-dependent variations were observed in nitrogen and fat absorption and in the retention of nitrogen in some groups of treated rats (Bhat and Chandrasekhara, 1986b). The results of this study show a no-observed-adverse effect level (NOAEL) of 20 mg/kg bw/day. In its safety assessment, JECFA also considered this dose as the NOAEL (WHO, 2006).

2.4. Immunotoxicity

Dogra et al. (2004) investigated the potential immunological effects of piperine. In this study, 6-week-old Swiss male mice (6/group; weight- 20 g) were gavaged at a dose of 0, 1.12, 2.25, or 4.5 mg/kg body weight/day for five consecutive days. Piperine administration had no overt toxic effect and "the liver gained weight normally." The highest dose of piperine resulted in significant decreases in the weights of spleen, thymus and mesenteric lymph nodes, but not of peripheral lymph nodes. Treatment with piperine at all dose levels suppressed the cellular population of lymphoid organs, except for the spleen, where the doses of 1.12 and 2.25 mg/kg bw caused an increase. Piperine at doses of 2.25 and 4.5 mg/kg bw caused a significant reduction in total leucocyte counts and an increase in the percentage of neutrophils (although results presented do not show statistical significance). At dose levels of 2.25 and 4.5 mg/kg bw, mitogenic response of B-lymphocyte to lipopolysaccharide was suppressed. The doses of 1.12 and 2.25 mg/kg bw suppressed the mitogenic response of T-lymphocytes to phytohemagglutinin and the nitroblue tetrazolium dye reducing activity of peritoneal exudate cells (PECs). The investigators determined the lowest dose of 1.12 mg/kg bw as the NOAEL. The results presented by these investigators do not show a dose-related effects of piperine as several parameters showed an increase at low dose and a decrease at high dose. The results of this study are difficult to interpret. Other studies using substantially higher doses of piperine (Bhat and Chandrasekhara, 1986b) did not reveal adverse effects, including effects on leucocyte count and spleen weight.

2.5. Genotoxicity Studies

Mutagenicity-related studies of piperine are summarized in Table 5. Piperine was found to be non-mutagenic in four test systems: *Salmonella typhimurium*, micronucleus, sperm morphology and dominant lethal assays (Karekar et al., 1996). In the Ames *Salmonella* test, six different doses of piperine, in the range of 0.005-10 μmol/plate, did not induce His^+ revertants in the presence or absence of metabolic activation. In the bone marrow micronucleus test in mice using two doses (10 and 20 mg/kg body weight),

piperine was non-mutagenic. As assessed by sperm morphology and dominant lethal tests in somatic cells, piperine (10 and 50 mg/kg body weight) failed to induce mutations in male germ cells of mice

In reverse mutation assays (Ames assay) employing different strains of *S. typhimurium* (Table 5), piperine did not cause mutagenic effects in the presence or absence of metabolic activation. Piperine did not induce micronuclei in the bone marrow of male Swiss mice following two intraperitoneal doses (at 0 and 24 hours) for a total dose of up to 4 mg/kg bw (Muralidhara and Narasimhamurthy, 1990). In another study, as assessed by sperm shape abnormality and tests for dominant lethal mutations in mice, piperine did not cause mutations in male germ cells. In different studies, mice given piperine at doses of up to 75 mg/kg bw/day by gavage or up to 4 mg/kg bw/day by intraperitoneal injection for up to 5 days showed no sperm shape abnormalities or dominant lethal mutations (Muralidhara and Narasimhamurthy, 1990; Karekar et al., 1996; Daware et al., 2000). The available evidence suggests that piperine is non-mutagenic.

Madrigal-Bujaidar et al. (1997) investigated mutagenic effects of an alcoholic extract of the mature berries of black pepper. The mutagenicity was studied by *in vivo* (mouse bone marrow assay) and *in vitro* (human lymphocytes). In both the assays the rate of sister chromatid exchange (SCE) and the replicative index were determined. For the *in vivo* study, mice were treated intraperitoneally with doses of 7, 14, 28 and 56 mg/kg bw. The results revealed a significant increase of SCE frequency at all doses tested compared with the control and a similar pattern with regard to cell proliferation kinetics at all doses tested, without significant differences between them. For the *in vitro* assay, doses of 25, 50, 75 and 100 µg/ml resulted in a significant increase in the frequency of SCEs at all doses tested and a significant reduction in the replicative index, at the two high doses. These results indicate that the extract of black pepper was genotoxic in both systems. Contrary to these findings, as described above piperine did not induce micronuclei in the bone marrow of male Swiss mice given a single dose of 10 or 20 mg/kg bw by gavage (Karek ar et al., 1996) or two intraperitoneal doses (at 0 and 24 hours) for a total dose of up to 4 mg/kg bw (Muralidhara & Narasimhamurthy, 1990). As BioPerine® primarily contains (>95%) piperine, the available evidence indicates that it is not mutagenic.

Table 5. Mutagenicity studies of piperine

Test system	Endpoint	Concentration	Result	Reference
S. typhimurium TA97a, TA98, TA100, TA102	Reverse Mutation	0.01, 0.5, or 10 µmol/plate (3, 143, or 2,853 µg/plate)	Negative (+/- S9)	Karekar et al., 1996
S. typhimurium TA97a, TA98, TA100, TA102	Reverse Mutation (pre incubation)	0.005, 0.05, 0.5, or 5 µmol/plate (1, 14, 143, or 1,427 µg/plate)	Negative (+/- S9)	Karekar et al., 1996
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse Mutation	1,000 µg	Negative (+/- S9)	Andrews et al., 1980
Male Swiss mice	Micronuclei (bone marrow)	10 or 20 mg/kg bw (single gavage)	Negative	Karekar et al., 1996
Male Swiss mice	Micronuclei (bone marrow)	1, 2, or 4 mg/kg bw (ip at 0 and 24 hours)	Negative	Muralidhara and Narasimhamurthy, 1990
Male Swiss mice	Sperm Morphology	10 or 50 mg/kg bw/day (gavage for 5 days)	Negative	Karekar et al., 1996
Male Swiss mice	Sperm Morphology	35, 50, or 75 mg/kg bw/day (orally for 5 days)	Negative	Daware et al., 2000
Male Swiss mice	Sperm Morphology	1, 2, or 4 mg/kg bw/day (ip for 5 days followed by 35 day maintenance)	Negative	Muralidhara and Narasimhamurthy, 1990
Male and female Swiss mice	Dominant Lethal Mutations	10 or 50 mg/kg bw (single gavage)	Negative	Karekar et al., 1996
Male Swiss mice	Dominant Lethal Mutations	4 mg/kg bw/day (ip for 5 days followed by 35 day maintenance)	Negative	Muralidhara and Narasimhamurthy, 1990
Male Swiss mice	Micronuclei (bone marrow)	75 mg/kg bw/day (orally for 5 days)	Negative	Selvendiran et al., 2005

Abbreviations- ip = intraperitoneal; bw = body weight

2.6. Carcinogenicity-Related Studies

In a chemoprevention study, Selvendiran et al. (2004) investigated the potential anti-carcinogenic potential of piperine in mice by measuring alterations of the mitochondrial antioxidant system and lipid peroxidation in benzo(a)pyrene-induced experimental lung carcinogenesis. In this study, five groups of male Swiss albino mice (10-12 week-old; weight not reported) were treated orally with vehicle (control, corn oil), benzo(a)pyrene (50 mg/kg bw weekly twice for 4 weeks), piperine+benzo(a)pyrene (during initiation), piperine+benzo(a)pyrene (post initiation), and piperine (alone, 50 mg/kg bw/day for 16 weeks). Oral supplementation of piperine (50 mg/kg bw/day) effectively suppressed lung carcinogenesis in benzo(a)pyrene-induced mice as revealed

by the decrease in the extent of mitochondrial lipid peroxidation and concomitant increase in the activities of enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) and non enzymatic antioxidant (reduced glutathione, vitamin E, and vitamin C) levels when compared to lung tumor bearing animals. Compared to the control group, mice treated with piperine alone did not show any significant changes in the activities of superoxide dismutase, catalase, and glutathione peroxidase, or in the levels of reduced glutathione, vitamin E, and vitamin C. These results suggest that oral administration of piperine alone at levels of 50 mg/kg bw/day for 16 weeks did not cause any adverse effects on the antioxidant activity.

2.7. Reproductive and Developmental Toxicity

In an *in vitro* study, Piyachaturawat et al. (1991) investigated the effect of piperine on the fertilizing ability of hamster sperm. In this study, sperm were incubated in a capacitation medium for 3 hours prior to co-incubation with hamster eggs in a fertilization medium for another 3 hours. Addition of 0.18–1.05 mM piperine reduced both the percentage of eggs fertilized and the degree of polyspermia in a dose-dependent manner. The concentration of piperine used in this *in vitro* study seems very high as it is unlikely that such high concentrations could be reached following oral administration of piperine.

Daware et al. (2000) studied the reproductive toxicity of piperine in adult female Swiss albino mice (30-35 g). In this study the effects of piperine on the estrous cycle, mating behavior, toxicity to male germ cells, fertilization, and the implantation and growth of pups were investigated. Females mice showing normal estrous cycles were treated (dose volume 10 ml/kg bw) orally with 0 (vehicle control; 1% CMC), 10 or 20 mg piperine/kg bw/day for 14 days and the estrous cycle pattern was studied throughout the period of treatment. After treatment the females were allowed to mate with normal males. The mating performance was determined from a vaginal plug or sperm-positive smear. Pregnant females were allowed to deliver and the growth of the pups was monitored up to 21 days post-partum. In addition to these experiments, relevant short-term tests were employed to assess the effect on the estrous cycle, mating behavior, toxicity to male germ cells, fertilization, implantation and growth of pups. Administration of piperine (10 and 20 mg/kg bw) increased the period of the diestrous phase (statistically not significant) which seemed to result in decreased mating performance and fertility. As regards possible effects on implantation, post-mating five days (day 1-5) oral treatment of piperine caused 83% inhibition of implantation at both 10 and 20 mg/kg bw. The post-implantation survival and sex ratio was not affected by piperine treatment. Piperine did not affect post-partum litter growth. Based on these observations, the investigators concluded that piperine interferes with several crucial reproductive events in a mammalian model. The article is quite confusing as the results of different experiments were described together to draw the conclusion. Also the reason for the lack of dose-related effects on implantation is not clear. It is difficult to explain the results of this study, as the study was not conducted according to standard guidelines.

2.8. Human Studies

As described earlier (Section 2.1.1. Metabolism), in some studies piperine has been shown to influence bioavailability of other substances. Shoba et al. (1998)

investigated the effects of piperine on the pharmacokinetics of curcumin. In human volunteers (n=10) receiving a single oral dose of 2 g curcumin, serum levels were either undetectable or very low. However, concurrent administration of curcumin with 20 mg piperine resulted in very high serum levels of curcumin from 0.25 to 1 hour post-dosing, and bioavailability of curcumin was increased by 20-fold. No toxicity was observed in the 10 subjects who participated in this study.

Based on a secondary citation (Srinivasan, 2007), Atal et al. (1981) attempted to investigate the scientific basis of the use of the "trikatu" group of acrids (long pepper, black pepper, and ginger) commonly found in a large number of prescriptions in the Indian Ayurvedic system of medicine. In this study, *P. longum* (long pepper) increased the blood levels of the test drug, vasicine, by nearly 233%. Similarly, piperine increased blood levels of the test drug, sparteine, by over 100%. These results suggest that the acrids have the capacity to increase the bioavailability of certain drugs. The authors suggested that the "trikatu" group increases the bioavailability of drugs either by promoting rapid absorption from the gastrointestinal tract, or by protecting the drug from being metabolized in its first passage through the liver after being absorbed, or by a combination of these two mechanisms. However, absent information regarding the doses of *P. longum* required to produce the reported effects, it is difficult to assign significance to these findings.

Based on the results of an *in vitro* study, Naidu and Thippeswamy (2002) reported that piperine was an effective antioxidant and offered protection against the oxidation of human low density lipoprotein (LDL) as evaluated by copper ion-induced lipid peroxidation of human LDL, which was measured based on the formation of thiobarbituric acid reactive substances and relative electrophoretic mobility of LDL on agarose gel. In another *in vitro* study, Prasad et al. (2004) investigated the effects of an aqueous extract of black pepper as well as piperine on human polymorphonuclear lymphocyte 5-lipoxygenase, the key enzyme involved in biosynthesis of leukotrienes. The formation of 5-lipooxygenase product 5-HETE was significantly inhibited in a concentration-dependent manner with IC_{50} values of 0.13 mg for aqueous extracts of pepper and 60 μM for piperine. The results of these studies indicate that piperine might exert an antioxidant physiological role by modulating 5-lipooxygenase pathway.

3. SUMMARY

Black pepper has a long history of use as a spice. In addition to its common use as a flavoring ingredient, black pepper is also used as a preservative, therapeutic agent, in perfumery, and as an insecticide. The ancient texts of the Indian traditional health care system, Ayurveda, describe the use of black pepper for a wide variety of health benefits. As a spice, it is valued for its distinct biting quality attributed to the pungent alkaloid piperine. Approximately 40% of the oleoresin of black pepper is piperine. The historical use of black pepper and its preparations in foods without reports of significant adverse effects suggests that human exposure to black pepper at dietary levels is without significant risk of harm. Use of black pepper, black pepper oil, and black pepper oleoresin as flavoring agents in foods has been recognized as GRAS by the FDA and FEMA. Both these agencies have also recognized the use of its active ingredient piperine. FDA has approved use of synthetic piperine as a food additive flavoring and adjuvant,

while FEMA has included piperine in its GRAS list. Additionally, JECFA has evaluated the use of piperine as a food additive and established an acceptable daily intake as "acceptable." The available information indicates that the average daily intake of piperine from its presence in black pepper is approximately 32 mg.

Sabinsa Corporation intends to use BioPerine®, a standardized extract prepared from the fruits of *P. nigrum* L (black pepper) or *P. longum* L (long pepper), as a flavoring ingredient at use levels of 15 ppm in Baked Goods, Breakfast Cereals, Milk Products, Cheese, Egg Products, Processed Vegetables, Soft Candy, Soups, Snack Foods, Beverages Type I - Non-alcoholic, Imitation Dairy Products, Hard Candy, Chewing Gum, Granulated Sugar, Seasonings & Flavors. BioPerine® manufactured according to current good manufacturing practices contains >95% piperine. The mean and 90th percentile all-user intake of BioPerine® from its intended food uses is estimated as 6.85 and 13.70 mg/person/day, respectively. The estimated 90th percentile intake (13.70 mg/day) of BioPerine® resulting from the intended use levels in specified foods is equivalent on a body weight basis to 0.23 mg/kg body weight/day for an adult weighing 60 kg. Because of its pungent taste similar to that of black pepper, BioPerine® and black pepper are unlikely to be used in the same foods. Hence the intake of piperine from the proposed uses of Bioperine® is unlikely to be additive to the existing piperine intake from the use of black pepper and pepper derivatives in food.

The available evidence suggests that following its oral ingestion, piperine is rapidly absorbed from the gastrointestinal tract. Irrespective of the route of administration, approximately 3% of the unchanged dose was detected in feces over 5 days. Piperine was distributed in different tissues and only traces were noted in blood. Piperine was excreted in urine as glucuronides, sulfates and phenols, with maximum excretion levels on days 1-4. Approximately 91-97% of the administered dose was accounted for. At high doses, piperine has been reported to inhibit metabolizing activity of several cytochrome P450 (CYP) isoforms, as well as glucuronidation. Available evidence suggest that piperine induced inhibition of CYPs is nonspecific and rapidly reversible. The alteration of metabolism by piperine remains to be fully investigated from a mechanistic perspective and is the subject of several current investigations.

In acute toxicity studies, the oral LD_{50} of piperine in rats and mice ranged from 330 to 514 mg/kg body weight. In a short-term dose response study in rats, no adverse effects of piperine at a dose of 100 mg/kg bw/day were noted. In a repeat-dose study, administration of black pepper, its oleoresin and piperine that provided average daily intakes of approximately 5, 10 and 20 mg/kg bw/day of piperine, no adverse effects were noted. JECFA derived a NOAEL of 20 mg/kg bw/day for piperine based on the results of this study. Based on the observations from a chemoprevention study, oral supplementation with piperine to rats at levels of 50 mg/kg bw/day for 16 weeks did not cause any adverse effects on antioxidant activity.

In a series of genotoxicity studies, piperine was non-mutagenic. In a poorly designed study, piperine was reported to affect reproductive events in a mammalian model. In this study, results of several different experiments were described to draw the conclusion. The article was quite confusing and no dose-related effects on implantation were noted. However, the available evidence from the history of common use of black pepper and its preparations did not reveal any reports of reproductive toxicity. In a dose-

related immunotoxicity study, the NOAEL of piperine was determined as 1.12 mg/kg bw; however, the data presented in the article do not show dose-related effects of piperine. In a review article Srinivasan (2007) stated that, "Although initially there were a few controversial reports regarding the safety of black pepper or piperine as a food additive, the later studies have established the safety of this spice in several animal studies." This conclusion is further supported by the regulatory reviews and approval of piperine as a flavoring agent.

The evidence of BioPerine® safety is supported by:

- Black pepper, its preparations and piperine have a long history of common food use.
- Humans have been regularly exposed to black pepper and thus in turn to piperine without reports of significant adverse effects at commonly used levels.
- There is no evidence that consumption of black pepper and its preparations either in foods or as dietary supplement have any cumulative effect and this observation is supported by the findings of pharmacokinetic studies.
- Experimental studies, including subchronic toxicity, *in vitro* and *in vivo* genotoxicity findings corroborate the safety-in-use of piperine.
- Regulatory agencies, including FDA, JECFA and FEMA have permitted use of piperine in foods.

There is sufficient qualitative and quantitative scientific evidence, including human and animal data, to determine safety-in-use or acceptable daily intake (ADI) for BioPerine®. Generally, ADIs are derived from a no-observed-adverse-effect level determined from animal studies with considerations of uncertainty factors to account for variability and uncertainties. Based on the results of a repeat-dose study of piperine, JECFA derived a NOAEL of 20 mg/kg bw/day. Given the common use of black pepper and its preparations, and approval of the use of piperine by the regulatory agencies, the use of an uncertainty factor of 100 (10 for intraspecies variation and 10 for interspecies variations) is considered appropriate. Using an uncertainty factor of 100 and the JECFA NOAEL of 20 mg/kg bw/day, the resulting ADI is determined as 0.2 mg/kg bw/day.. For an individual weighing 60 kg the ADI for piperine is 12 mg/person/day. Although there are some reports from experimental studies claiming adverse effects of piperine, these studies do not appear to be conducted as per the standard guidelines for such type of studies. Additionally, the fact that piperine has been consumed as a principal constituent of pepper in the diet and is approved by agencies such as FDA and JECFA, supports the safety in use of piperine.

The safety determination of BioPerine® is based on the totality of evidence, including current approved uses of piperine, human observations, and animal studies. The 90^{th} percentile intake of 13.70 mg BioPerine®/person/day is similar to the safe levels (12 mg/day) determined on the basis of available safety studies. The proposed intake is 2-3 times lower than the background estimated daily intake (32 mg/day) of piperine from its presence in black pepper. The proposed intake of piperine from BioPerine® is unlikely to add to the background intake from black pepper or its oleoresin as the principal constituent piperine is known for its strong pungent taste. Further, the strong pungent

taste, BioPerine® is unlikely to be added to foods that already contain black pepper or its oleoresin. On the basis of scientific procedures[4], and history of exposure from natural dietary sources, the consumption of BioPerine® as an added food ingredient is considered safe at use levels up to 15 ppm (15 mg/kg). The intended uses are compatible with current regulations, *i.e.,* BioPerine® is used in specified foods (described in this document) and is produced according to current good manufacturing practices (cGMP).

[4] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

4. CONCLUSION

Based on a critical scientific and safety evaluation of the publicly available data summarized herein, the Expert Panel members whose signatures appear below have individually and collectively concluded that BioPerine®, produced according to current Good Manufacturing Practices (cGMP) and meeting the specifications cited herein, when used as a flavoring agent [21 CFR§170.3(o)(12)] at maximum use levels of up to 15 ppm (15 mg/kg) in specific foods (Baked Goods, Breakfast Cereals, Milk Products, Cheese, Egg Products, Processed Vegetables, Soft Candy, Soups, Snack Foods, Beverages Type I - Non-alcoholic, Imitation Dairy Products, Hard Candy, Chewing Gum, Granulated Sugar, Seasonings & Flavors) described in this monograph and resulting in a 90th percentile estimated intake of 13.70 mg BioPerine®/person/day is safe.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that BioPerine®, when used as described, is GRAS based on scientific procedures.

Signatures

(b) (6)

John A. Thomas, Ph.D., F.A.C.T., D.A.T.S. — Date 5/14/13

(b) (6)

James T. Heimbach, Ph.D., F.A.C.N. — Date May 10, 2013

(b) (6)

Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S. — Date May 17, 2013

5. REFERENCES

Andrews, A.W., Fornwald, J.A., Lijinsky, W., 1980. Nitrosation and mutagenicity of some amine drugs. Toxicol. Appl. Pharmacol. 52:237-244.

Atal, C.K., Dubey, R.K., Singh, J., 1985. Biochemical basis of enhanced drug bioavailability by piperine: evidence that piperine is a potent inhibitor of drug metabolism. J. Pharmacol. Exp. Ther. 232: 258-262.

Atal, C.K., Zutshi, U., Rao, P.G., 1981. Scientific evidence on the role of Ayurvedic herbals on bioavailability of drugs. J. Ethnopharmacol. 4:229-232 (cited in Srinivasan, 2007).

Badmaev, V., Majeed, M., Prakash, L., 1999. Piperine, an alkaloid derived from black pepper increases serum response of beta-carotene during 14-days of oral beta-carotene supplementation. Nutr. Res. 19:381-388.

Badmaev, V., Majeed, M., Prakash, L., 2000. Piperine derived from black pepper increases the plasma levels of coenzyme Q10 following oral supplementation. J. Nutr. Biochem. 11:109-113.

Bano, G., Raina, R.K., Zutshi, U., Bedi, K.L., Johri, R.K., Sharma, S.C., 1991. Effect of piperine on bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers. Eur. J. Clin. Pharmacol. 41:615-617.

Bhardwaj, R.K., Glaeser, H., Becquemont, L., Klotz, U., Gupta, S.K., Fromm, M.F., 2002. Piperine, a major constituent of black pepper, inhibits human P-glycoprotein and CYP3A4. J. Pharmacol. Exp. Ther. 302:645-650.

Bhat, B.G., Chandrasekhara, N., 1986a. Studies on the metabolism of piperine: Absorption, tissue distribution and excretion of urinary conjugates in rats. Toxicol. 40:83-92.

Bhat, B.G., Chandrasekhara, N., 1986b. Lack of adverse effects of black pepper, its oleoresin and piperine in weanling rat. J. Food Safety 7:215-223.

Bhat, B.G., Chandrasekhara, N., 1987. Metabolic disposition of piperine in rat. Toxicol. 44:99-106.

Bajad, S., Singh, A.K., Bedi, K.L., 2002. L iquid chromatographic method for determination of piperine in rat plasma: application to pharmacokinetics. J. Chromatography B 776: 245-249.

Bray, H.G., James, S.P., Thorpe, W.V., Wasdell, M.R., Wood, P.B., 1949. The fate of certain organic acids and amides in the rabbit. 9. Lower aliphatic amides. Biochem. J. 45:467-471.

Daware, M.B., Mujumdar, A.M., Ghaskadbi, S., 2000. Reproductive toxicity of piperine in Swiss albino mice. Planta Med. 66:231-236.

DiNovi, M.J., Kuznesof, P.M., 1995. Estimating Exposure To Direct Food Additives And Chemical Contaminants in the Diet. Available at http://www.cfsan.fda.gov/~dms/opa-cg8.html Website visited: March 28, 2013.

Dogra, R.K., Khanna, S., Shanker, R., 2004. Immunotoxicological effects of piperine in mice. Toxicology 196:229-236.

DSHEA, 1994. Dietary Supplements Health and Education Act of 1994. US Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, DC.

EAFUS, 2008. Everything Added to Food in the United States (EAFUS). Complete list of substances available at: http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=eafusListing&displayAll=true (website visited on March 29, 2013).

FASEB, 1988. Estimation of Exposure to Substances in the Food Supply, Anderson, S.A., Editor, FDA Contract No. 223-84-2059, Life Sciences Research Office, Bethesda, MD.

FDA, 2006. Food and Drug Administration. *Estimating Dietary Intake of Substances in Food.* Guidance document released August 2006. Document available at: http://www.fda.gov/food/guidancecomplianceregulatoryinformation/guidancedocuments/foodingredientsandpackaging/ucm074725.htm (website visited on March 27, 2013)

Fenroli, G., 1974. Piperine. Fenaroli's Handbook of Flavor Ingredients 2nd Ed. (1975) CRC Press, Cleveland, Ohio, U.S.A.

Gopalam, A., Zacharia, T. J., 1988. Black pepper oil - chemical and aroma quality. Indian Cocoa, Arecanut & Spices J. 12:44-45.

Govindarajan, V. S., 1977. Pepper - Chemistry, technology, and quality evaluation. CRC Crit. Rev. Food Sci. Nutr. 9:115-225.

Hall, R.L., Oser, B.L., 1965. Recent progress in the consideration of flavoring ingredients under the food additives amendment III. GRAS substances. Food Technol. 19:151-197.

JECFA, 2005. Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives. Piperine. Last updated 31 Mar 06. Available online from: http://www.inchem.org/documents/jecfa/jeceval/jec_1931.htm (site visited on February 26, 2013).

Kang, M.H., Won, S.M., Park, S.S., Kim, S.G., Novak, R.F., Kim, N.D., 1994. Piperine effects on the expression of P4502E1, P4502B and P4501A in rat. Xenobiotica 24:1195-1204.

Karekar, V. R., Mujumdar, A. M., Joshi, S. S., Dhuley, J., Shinde, S. L., Ghaskadbi, S., 1996. Assessment of genotoxic effect of piperine using *Salmonella typhimurium* and somatic and somatic and germ cells of Swiss albino mice. Arzneimittelforschung 46:972-975.

Khajuria, A., Zutshi, U., Bedi, K.L., 1998. Permeability characteristics of piperine on oral absorption–an active alkaloid from peppers and a bioavailability enhancer. Indian J. Exp. Biol. 36: 46-50.

Kindell, S.S., 1984. Studies on selected genotoxic and toxic properties of piperine. Ph.D. thesis, Drexel University, Philadelphia, PA. (Cited in Bhardwaj et al., 2002).

Lambert, J.D., Hong, J., Kim, D.H., Mishin, V.M., Yang, C.S., 2004. Piperine enhances the bioavailability of the tea polyphenol (–)-epigallocatechin-3-gallate in mice. J. Nutr. 134:1948-1952.

Madrigal-Bujaidar, E., Diaz Barriga, S., Mota, P., Guzman, R., Cassani. M., 1997. Sister chromatid exchanges induced *in vitro* and *in vivo* by an extract of black pepper. Food Chem Toxicol. 35(6):567-571.

Marchetti, S., Mazzanti, R., Beijnen, J.H., Schellens, J.H., 2007. Concise review: Clinical relevance of drug drug and herb drug interactions mediated by the ABC transporter ABCB1 (MDR1, P-glycoprotein).Oncologist 12:927-941.

MRCA, 1965. Market Research Corporation of America (MRCA), in conjunction with the "Food intake and nutritive value of the diets of men, women and children in United States, Spring 1965". A Preliminary Report by the Consumer and Food Economics Research Division, Agricultural Research Division. United States Department of Agriculture, Washington, DC.

Muralidhara, Narasimhamurthy, K., 1990. Lack of genotoxic effects of piperine, (the active principle of black pepper) in albino mice. J. Food Safety 11:39-48.

Naidu, K.A., Thippeswamy, N.B., 2002. Inhibition of human low density lipoprotein oxidation by active principles from spices, Mol. Cell. Biochem. 229:19-23. (Cited in Srinivasan, 2007).

Pattanaik, S., Hota, D., Prabhakar, S., Kharbanda, P., Pandhi, P., 2006. Effect of piperine on the steady-state pharmacokinetics of phenytoin in patients with epilepsy. Phytother. Res. 20:683-686.

Piyachaturawat, P., Sriwattane W., Damrongphol, P., Pholpramool, C., 1991. Effects of piperine on hamster sperm capacitation and fertilization *in vitro*. Int. J. Androl. 14:283-290.

Piyachaturawat, P., Glinsukon, T., Toskulkao, C., 1983. Acute and subacute toxicity of piperine in mice, rats and hamsters. Toxicol. Lett. 16:351-359.

Prasad, N.S., Raghavendra, R., Lokesh, B.R., Naidu, K.A., 2004. Spice phenolics inhibit human PMNL5-lipoxygenase. Prostaglandins Leukot. Essent. Fatty Acids 70:521-528. (Cited in Srinivasan, 2007).

Ramasarma, T., 2000. Some radical queries. Toxicology 148:85-91.

Sabinsa Corporation (2013). Information on General description, specifications, composition and manufacturing of BioPerine® provided by Sabinsa Corporation.

Selvendiran, K., Banu, S.M., Sakthisekaran, D., 2005. Preliminary study on inhibition of genotoxicity by piperine in mice. Fitoterapia 76:296-300.

Selvendiran, K., Selvendiran, P., Magesh, V., Sakthisekaran, D., 2004. Modulatory effect of piperine on mitochondrial antioxidant system in Benzo(a)pyrene-induced experimental lung carcinogenesis. Phytomedicine 11:85-89.

Shoba, G., Joy, D., Thangam, J., Majeed, M., Rajendran, R., Srinivas, P.S.S.R., 1998. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Medica 64:353-356.

Singh, A., Dugga. S., 2009. Piperine- Review of advances in pharmacology. International J. Pharma. Sci. Nanotech. 2:615-620.

Singh, J., Dubey, R.K., Atal, C.K., 1986. Piperine-mediated inhibition of glucuronidation activity in isolated epithelial cells of the guinea-pig small intestine: evidence that piperine lowers the endogeneous UDP-glucuronic acid content. J. Pharmacol. Exp. Ther. 236:488-493.

Srinivasan, K., 2007. Black pepper and its pungent principle-piperine: a review of diverse physiological effects. Crit. Rev. Food Sci. Nutr. 47:735-748

Srinivasan, M. R., Satyanarayana, M. N., 1981. Effect of black pepper (*Piper nigrum* Linn.) and piperine on growth, blood constituents and organ weights in rats. Nutr. Rep. Int. 23:871–876. (Cited in Srinivasan, 2007).

Volak, L.P., Ghirmai, S., Cashman, J.R., Court, M.H., 2008. Curcuminoids inhibit multiple human cytochromes P450, UDP-glucuronosyltransferase, and sulfotransferase enzymes, whereas piperine is a relatively selective CYP3A4 inhibitor. Drug Metab. Dispos. 36:1594-1605.

Volak, L.P., Hanley, M.J., Masse, G., Hazarika, S., Harmatz, J.S., Badmaev, V., Majeed, M., Greenblatt, D.J., Court, M.H., 2013. Effect of a herbal extract containing curcumin and piperine on midazolam, flurbiprofen and paracetamol (acetaminophen) pharmacokinetics in healthy volunteers. Br. J. Clin. Pharmacol. 75(2):450-462.

Wilkinson, G.R., 1997. The effects of diet, aging and disease-states on presystemic elimination and oral drug bioavailability in humans. Adv. Drug Deliv. Rev. 27:129-159.

WHO, 2006. WHO Technical Report Series 934. Evaluation of Certain Food Additives. 65th report of JECFA. Aliphatic and aromatic amines and amides (Piperine). Report available online at: http://whqlibdoc.who.int/trs/WHO_TRS_934_eng.pdf (site visited on March 27, 2013).

6. APPENDIX I

Analytical data of BioPerine® from different lots (Sabinsa, 2013)

Specifications of BioPerine® from five different manufacturing batches

Parameter	Standard*	Lot # C80183E/H	Lot # C81162E/H	Lot # C81842E/H	Lot # C81993E/H	Lot # G80295E/H
Description	Off white to pale yellow powder	Complies	Complies	Complies	Complies	Complies
Identification	Comply with HPLC and IR spectrum	Complies	Complies	Complies	Complies	Complies
Solubility	Freely soluble in chloroform, soluble in alcohol, benzene and in glacial acetic acid. Insoluble in water	Complies	Complies	Complies	Complies	Complies
Loss on drying	NMT 2%	0.14	0.36	0.26	0.17	0.23
Melting range	Melts between 126°C to 132°C	126 to 130°C	128 to 130°C	128 to 131°C	128 to 130°C	127 to 128°C
Residue on ignition	NMT 0.1%	0.06	0.04	0.10	0.01	0.04
Tapped bulk density	Between 0.55 and 0.85 g/mL	0.63	0.63	0.56	0.57	0.58
Loose bulk density	Between 0.30 and 0.65 g/mL	0.44	0.33	0.47	0.41	0.43
Sieve test (passes through)						
- 20 mesh	NLT 100%	100	100	100	100	100
- 40 mesh	NLT 98%	100	100	100	100	100
- 80 mesh	NLT 95%	100	99.5	100	100	100
Assay						
Piperine content, HPLC	NLT 95 to 102% on dry basis	98	98	95.54	96.37	97.9
Heavy metals**	<10 ppm	<10	<10	<10	<10	<10
Arsenic	NMT 1 ppm	<0.2	<0.2	<0.2	<0.51	<0.2
Lead	NMT 3 ppm	2	1.12	2.15	1.34	<2
Microbiological						
Total plate count	< 3000 cfu/g	<150	<100	<100	<150	<100
Yeast and Mold	< 100 cfu/g	< 10	<10	<20	<10	15
Escherichia coli	Negative (cfu/10g)	Absent	Absent	Absent	Absent	Absent
Salmonella	Negative (cfu/10 g)	Absent	Absent	Absent	Absent	Absent
Staphylococcus aureus	Negative (cfu/10g)	Absent	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Negative (cfu/10g)	Absent	Absent	Absent	Absent	Absent
*Standard specifications for marketed product; **Heavy metal analysis performed by AAS or ICP-OES method.						

6.1. APPENDIX II

Residual solvent levels (ppm) from different batches of BioPerine®

Batch No.	Ethanol	Hexane	Ethyl acetate	Methanol
C100084E	56.48	3.16	56.91	ND
C91444E	88.39	ND	52.23	6.32
C91234E	66.4	8.1	50.1	11.6
C90874E	57.70	9.77	66.58	ND

ND = None detected

Shepherd, Lillian

From: MADHUSUDAN SONI <sonim@bellsouth.net>
Sent: Sunday, June 02, 2013 6:14 AM
To: Ramos-Valle, Moraima
Cc: Shepherd, Lillian; sonim@bellsouth.net
Subject: Re: GRAS Submission for Black Pepper Extract

Dear Dr. Ramos-Valle,
For the Black Pepper Extract GRAS, please note that Sabinsa Corporation has decided to exclude the egg products and any other food categories that fall under USDA jurisdiction. By oversight the egg product category was included in the submission.
Please note that currently, I am visiting India and will be back in the US on June 15. If you have any question or need additional information, please send me an email. I will try to call you tomorrow (Monday).
Best regards
Madhu

From: Shepherd, Lillian <Lillian.Shepherd@fda.hhs.gov>;
To: sonim@bellsouth.net <sonim@bellsouth.net>;
Subject: GRAS Submission for Black Pepper Extract
Sent: Wed, May 29, 2013 6:58:57 PM

Dear Dr. Soni,

This message is to confirm our receipt of your GRAS submission for Black Pepper Extract dated May 20, 2013. While looking at your submission for filing we noted that under the conditions of intended use section egg products are included. We seek clarification as to whether the egg products included in your intended use fall under USDA jurisdiction. Feel free to e-mail or call Moraima Ramos-Valle at (240) 402-1248 or Moraima.Ramos-Valle@fda.hhs.gov to further discuss your submission.

Thank you,

Lillian Shepherd

Lillian Shepherd

Program Analyst

240-402-1016

000040

Lillian.Shepherd@fda.hhs.gov

Shepherd, Lillian

From: Ramos-Valle, Moraima
Sent: Tuesday, June 04, 2013 2:39 PM
To: MADHUSUDAN SONI
Cc: Shepherd, Lillian
Subject: RE: GRAS Submission for Black Pepper Extract

Dear Dr. Soni,

Thanks for your response. We will attach this email to the submission, juts to make note that "egg products" are excluded. On your return you may send a more formal letter stating the exclusion of that food category from the intended use.

Enjoy your visit and have a safe return.

Moraima

Moraima J. Ramos Valle, M.S.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Phone: (240) 402-1248
Email: Moraima.Ramos-Valle@fda.hhs.gov

From: MADHUSUDAN SONI [mailto:sonim@bellsouth.net]
Sent: Tuesday, June 04, 2013 12:04 AM
To: Ramos-Valle, Moraima
Cc: Shepherd, Lillian; sonim@bellsouth.net
Subject: Re: GRAS Submission for Black Pepper Extract

Dear Dr. Ramos-Valle,
For the Black Pepper Extract GRAS, please note that Sabinsa Corporation has decided to exclude the egg products and any other food categories that fall under USDA jurisdiction. By oversight the egg product category was included in the submission.
Please note that currently, I am visiting India and will be back in the US on June 15. If you have any question or need additional information, please send me an email. I will try to call you tomorrow (Monday).
Best regards
Madhu

From: Shepherd, Lillian <Lillian.Shepherd@fda.hhs.gov>;
To: sonim@bellsouth.net <sonim@bellsouth.net>;
Subject: GRAS Submission for Black Pepper Extract
Sent: Wed, May 29, 2013 6:58:57 PM

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This message is to confirm our receipt of your GRAS submission for Black Pepper Extract dated May 20, 2013. While looking at your submission for filing we noted that under the conditions of intended use section egg products are included. We seek clarification as to whether the egg products included in your intended use fall under USDA jurisdiction. Feel free to e-mail or call Moraima Ramos-Valle at (240) 402-1248 or Moraima.Ramos-Valle@fda.hhs.gov to further discuss your submission.

Thank you,

Lillian Shepherd

Lillian Shepherd

Program Analyst

240-402-1016

Lillian.Shepherd@fda.hhs.gov

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SUBMISSION END